

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of the Claims:

1. – 39. (Cancelled)

40. (Currently Amended) A eukaryotic cell comprising an orthogonal aminoacyl-tRNA synthetase (O-RS), wherein the O-RS preferentially aminoacylates an orthogonal tRNA (O-tRNA) with at least one unnatural amino acid in the eukaryotic cell, which unnatural amino acid comprises p-propargyloxypheylalanine or p-azido-L-phenylalanine~~an alkynyl moiety or an azido moiety~~, wherein:

- (a) the O-RS or a portion thereof is encoded by a polynucleotide sequence as set forth in any one of SEQ ID NO.: 20-35;
- (b) the O-RS comprises an amino acid sequence as set forth in any one of SEQ ID NO.: 48-63; or,
- (c) the O-RS comprises an amino acid sequence that is at least ~~90%~~ 98% identical to that of a naturally occurring tyrosyl aminoacyl-tRNA synthetase (TyrRS) and comprises two or more amino acids selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37 of E. coli TyrRS; aspartate at a position corresponding to Asn126 of E. coli TyrRS; asparagine at a position corresponding to Asp182 of E. coli TyrRS; alanine, or valine, at a position corresponding to Phe183 of E. coli TyrRS; and, methionine, valine, cysteine, or threonine, at a position corresponding to Leu186 of E. coli TyrRS.

41. (Currently Amended) The cell of claim 40, further comprising an orthogonal tRNA (O-tRNA), wherein the O-tRNA recognizes a selector codon and is preferentially aminoacylated with the at least one unnatural amino acid by the O-RS, wherein the O-tRNA ~~is produced in a cell by cellular processing of~~ is encoded by a nucleic acid corresponding to SEQ ID NO.:65 and

produced in the cell, and the O-RS comprises a polypeptide sequence selected from the group consisting of: SEQ ID NO.: 48-63.

42. (Currently Amended) A polypeptide selected from the group consisting of:

- (a) a polypeptide that comprises an amino acid sequence as shown in any one of SEQ ID NO.: 48-63;
- (b) a polypeptide that comprises an amino acid sequence encoded by a polynucleotide sequence as shown in any one of SEQ ID NO.: 20-35;
- (c) a polypeptide that is specifically immunoreactive with an antibody specific for a polypeptide of (a), or (b);
- (d) a polypeptide that comprises an amino acid sequence that is at least ~~90%~~ 98% identical to that of a naturally occurring tyrosyl aminoacyl-tRNA synthetase (TyrRS) and comprises two or more amino acids selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37 of E. coli TyrRS; aspartate at a position corresponding to Asn126 of E. coli TyrRS; asparagine at a position corresponding to Asp182 of E. coli TyrRS; alanine, or valine, at a position corresponding to Phe183 of E. coli TyrRS; and, methionine, valine, cysteine, or threonine, at a position corresponding to Leu186 of E. coli TyrRS; and,
- ~~(e) a polypeptide that comprises at least 20 contiguous amino acids of SEQ ID NO.: 48-63 and two or more amino acid substitutions selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37 of E. coli TyrRS, aspartate at a position corresponding to Asn126 of E. coli TyrRS, asparagine at a position corresponding to Asp182 of E. coli TyrRS, alanine, or valine, at a position corresponding to Phe183 of E. coli TyrRS, and methionine, valine, cysteine, or threonine, at a position corresponding to Leu186 of E. coli TyrRS.~~

43. (Original) A composition comprising the polypeptide of claim 42 and an excipient.

44.- 46. (Cancelled)

47. **(Currently Amended)** A polynucleotide selected from the group consisting of:
- (a) a polynucleotide comprising a nucleotide sequence as set forth in any one of SEQ ID NO.: 20-35;
 - (b) a polynucleotide that is complementary to or that encodes a polynucleotide sequence of (a);
 - (c) a polynucleotide encoding a polypeptide that comprises an amino acid sequence as set forth in any one of SEQ ID NO.: 48-63;
 - (d) a polynucleotide that encodes a polypeptide of claim 42;
 - (e) a nucleic acid that hybridizes to a polynucleotide of (a), (b), (c), or (d) under highly stringent conditions over ~~substantially~~ the entire length of the nucleic acid;
 - (f), a polynucleotide that encodes a polypeptide that comprises an amino acid sequence that is at least ~~90%~~ 98% identical to that of a naturally occurring tyrosyl aminoacyl-tRNA synthetase (TyrRS) and comprises two or more mutations selected from the group consisting of: glycine, serine, or alanine at a position corresponding to Tyr37 of E. coli TyrRS, aspartate at a position corresponding to Asn126 of E. coli TyrRS, asparagine at a position corresponding to Asp182 of E. coli TyrRS, alanine, or valine, at a position corresponding to Phe183 of E. coli TyrRS, and methionine, valine, cysteine, or threonine, at a position corresponding to Leu186 of E. coli TyrRS; and,
 - (g) a polynucleotide that is at least 98% identical to a polynucleotide of (a), (b), (c), (d), (e), or (f).
48. **(Original)** A vector comprising a polynucleotide of claim 47.
49. **(Original)** The vector of claim 48, wherein the vector comprises a plasmid, a cosmid, a phage, or a virus.
50. **(Original)** The vector of claim 48, wherein the vector is an expression vector.
51. **(Original)** A cell comprising the vector of claim 48.
52. – 61. **(Cancelled)**